

Presented at the BIG4 Workshop
"3D Imaging and Innovative Approaches in Biosystematics"
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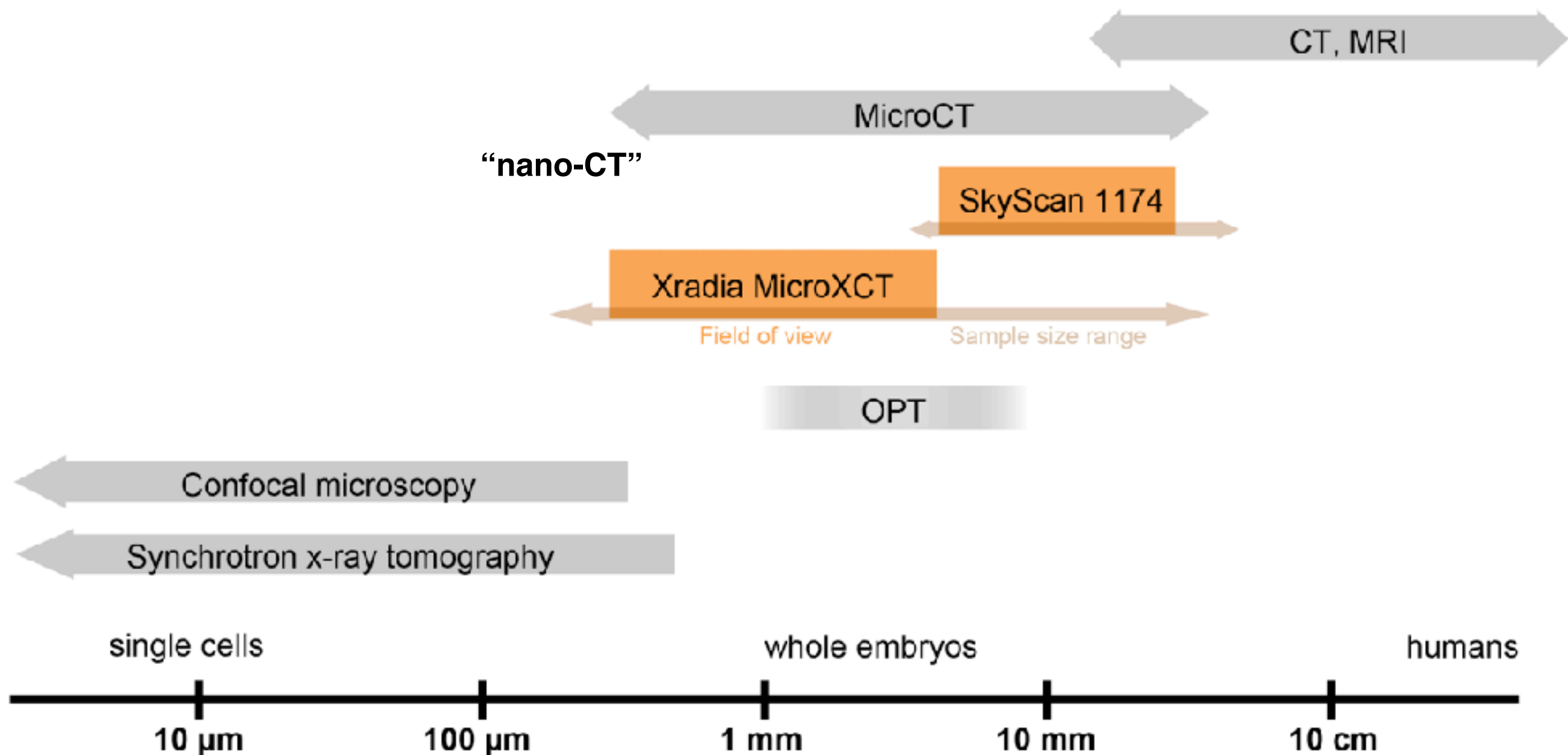


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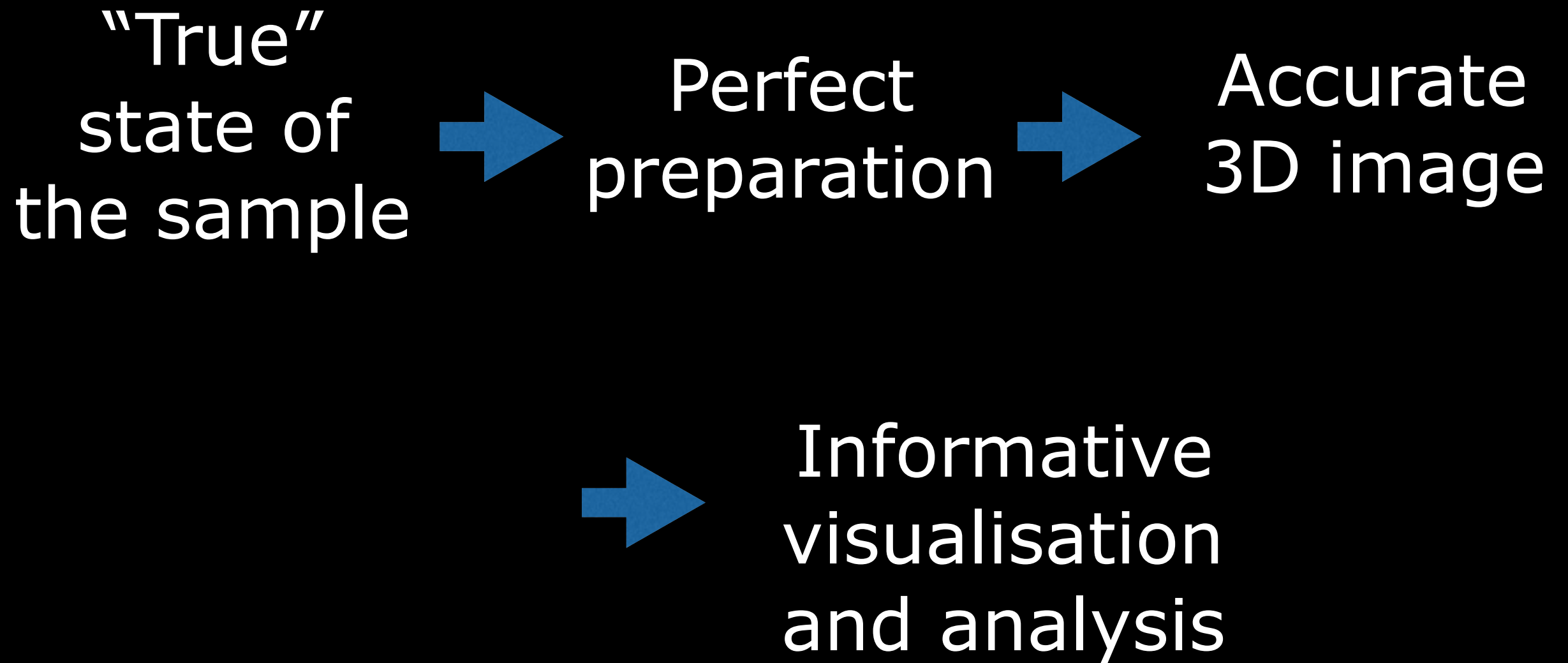
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It all starts with the sample



It all starts with the sample

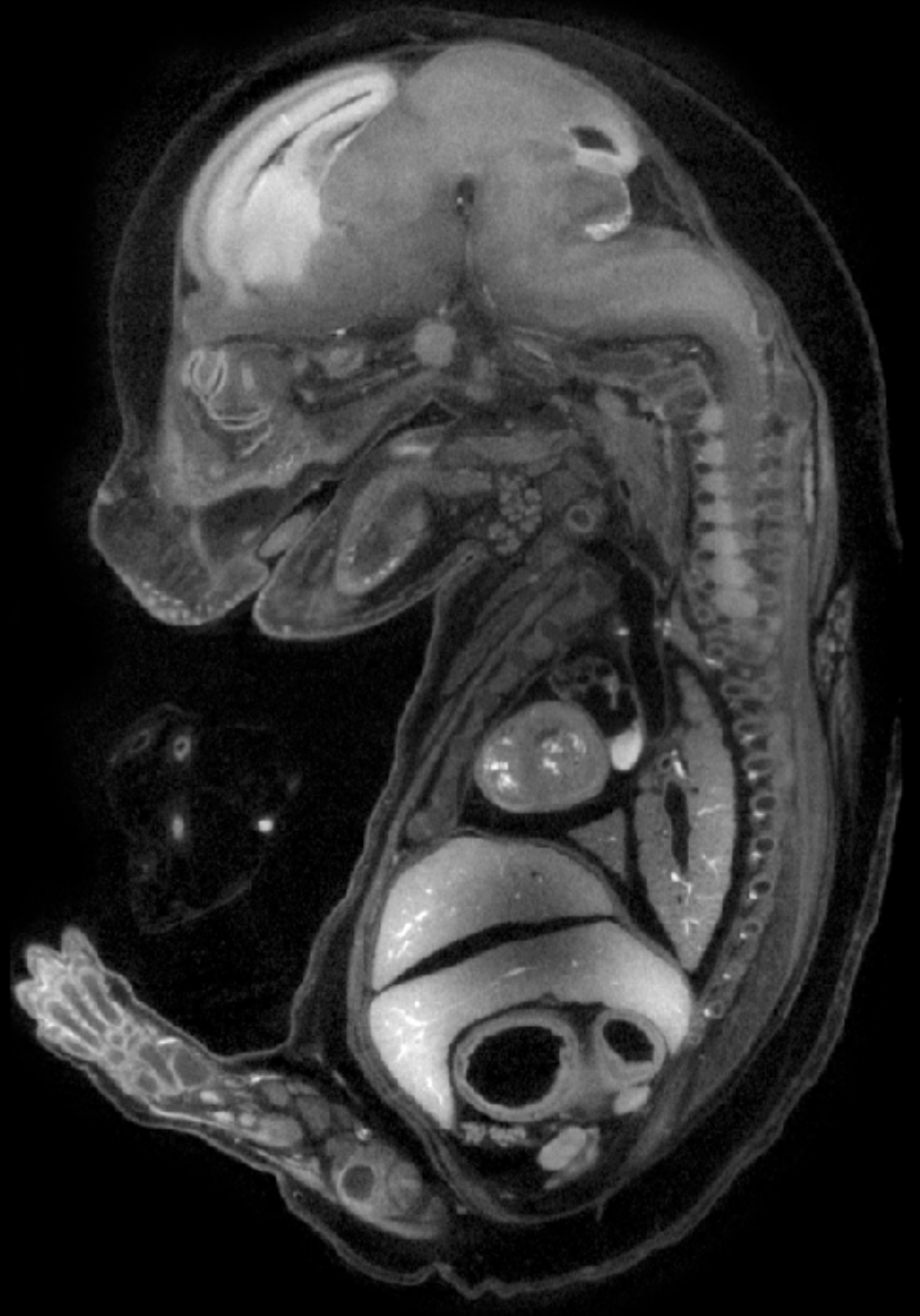


The quest for perfect soft tissue preservation

Stains cause
shrinkage

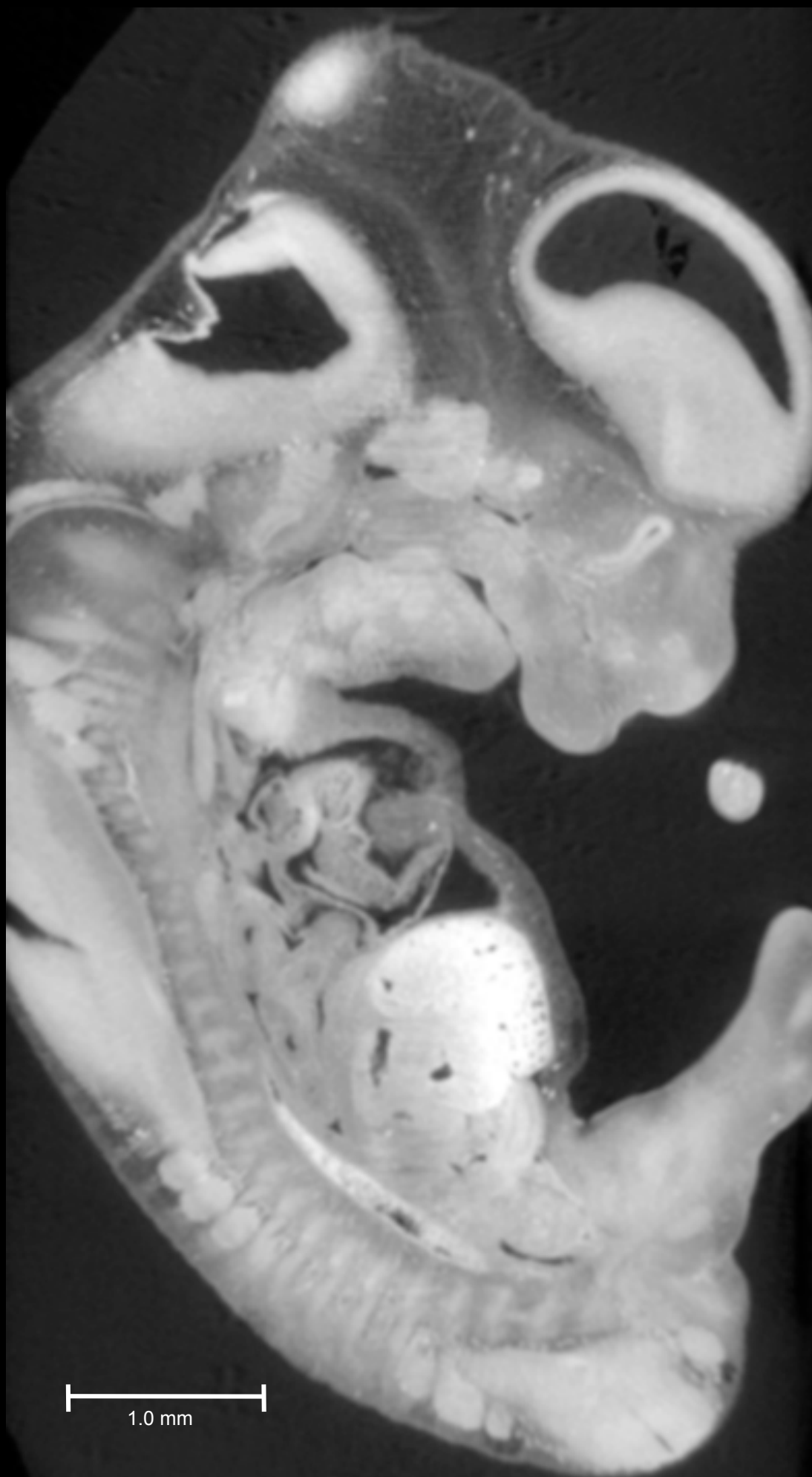
Polymer gel
provides support

Rat embryo, 18d



2001: The first
contrast-enhanced
microCT image
of an embryo

12-day mouse,
routine TEM prep:
osmium stained,
JB4 embedded;
scanned at the Mayo Clinic.
8 μ m voxels.



How to get x-ray contrast in soft tissues

Native mass-density differences

Phase methods

Condensation

Negative-space contrast

Contrast stains

- higher-Z agents bound or associated



*There are plenty
of good pieces
waiting to be
written in
C major.*

- Arnold
Schoenberg

12-day mouse,
tungsten
contrasted,
8 μ m voxels.

Raising the Z: contrast staining for soft biological materials



Chick embryo
St. 12, PTA



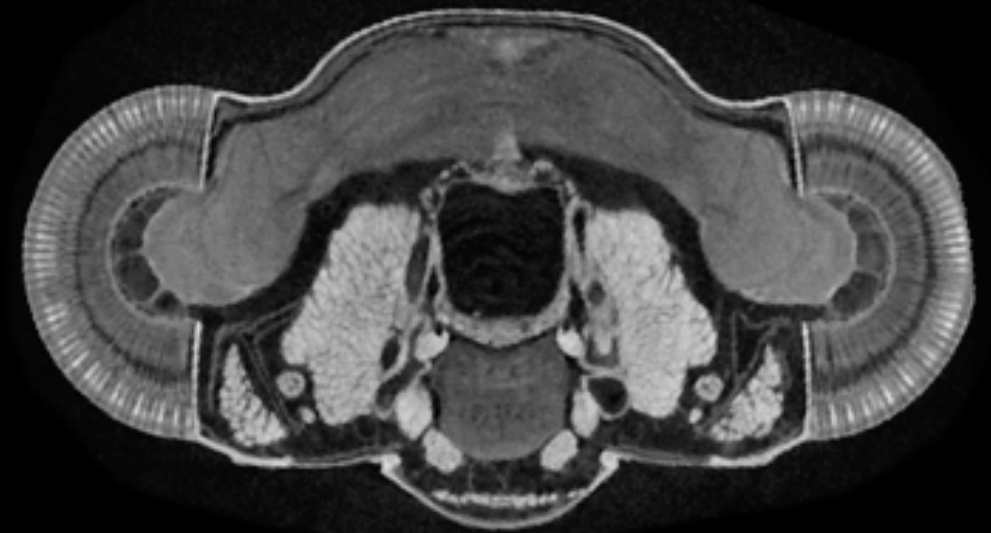
Chick somites
1 μm voxels

Metscher 2009,
BMC Physiol. 9:11,
Devel Dynam 238:632

Iodine works for arthropods

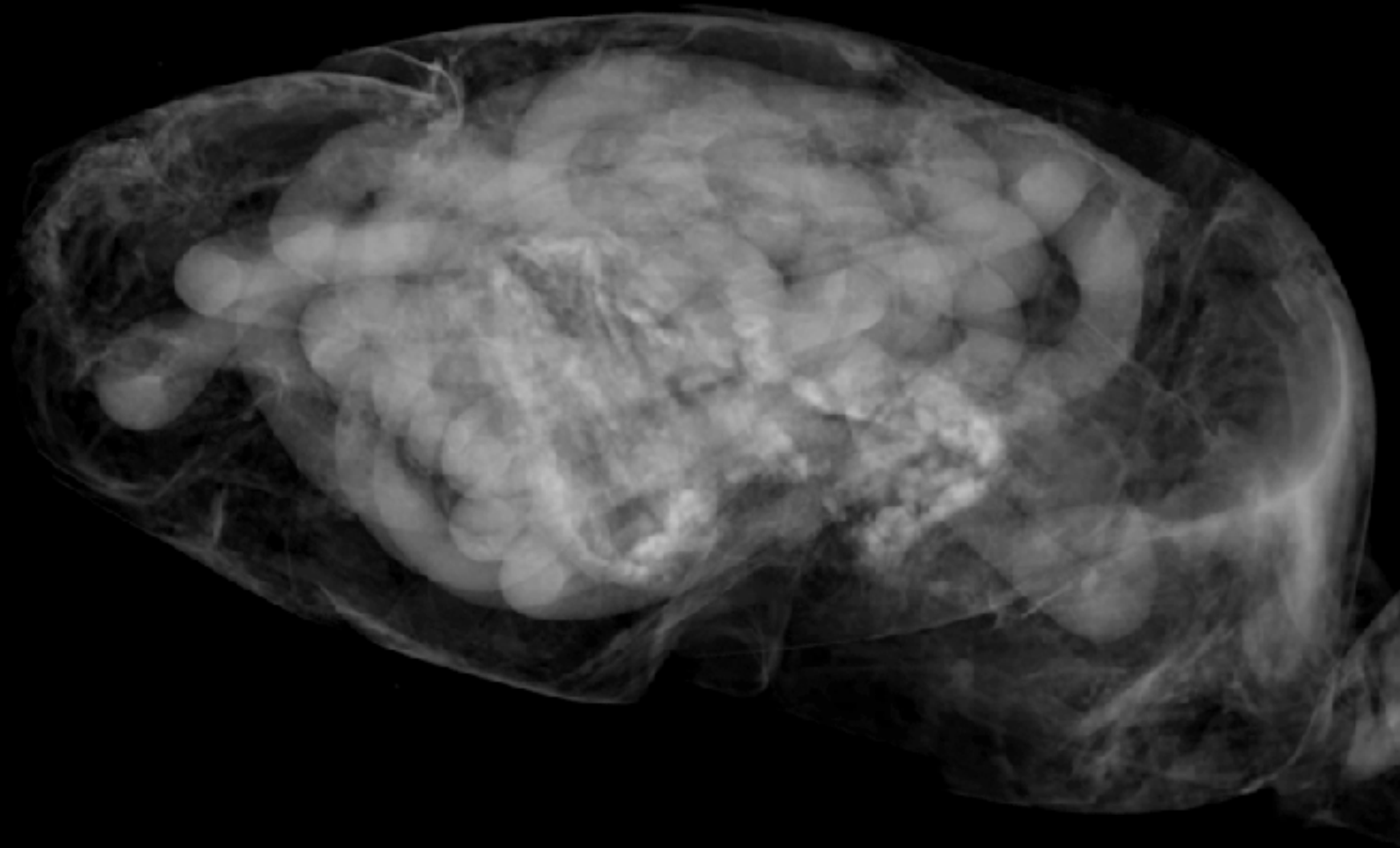
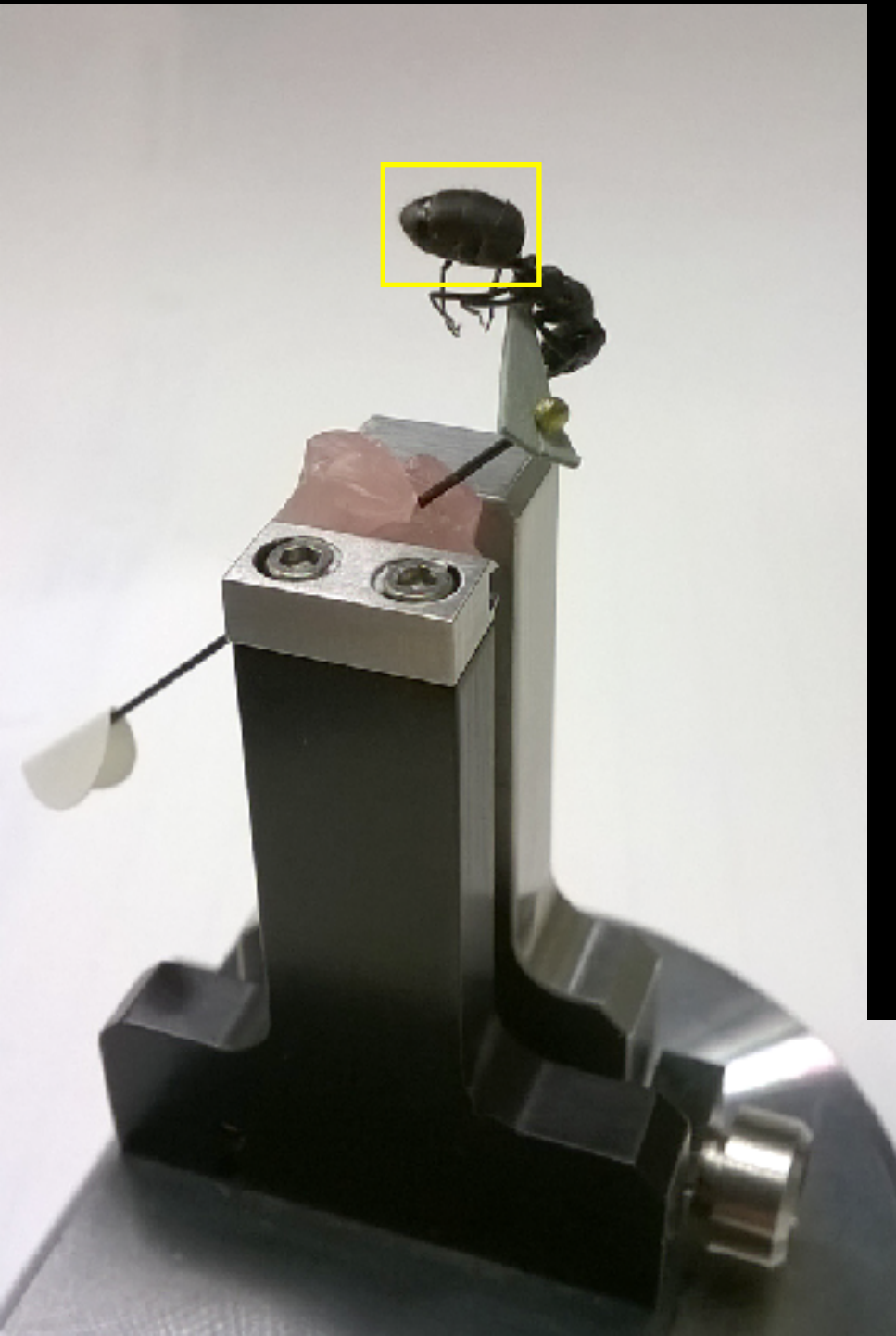


Iodine-enhanced microCT imaging has revealed that *Drosophila melanogaster* is actually a real insect.

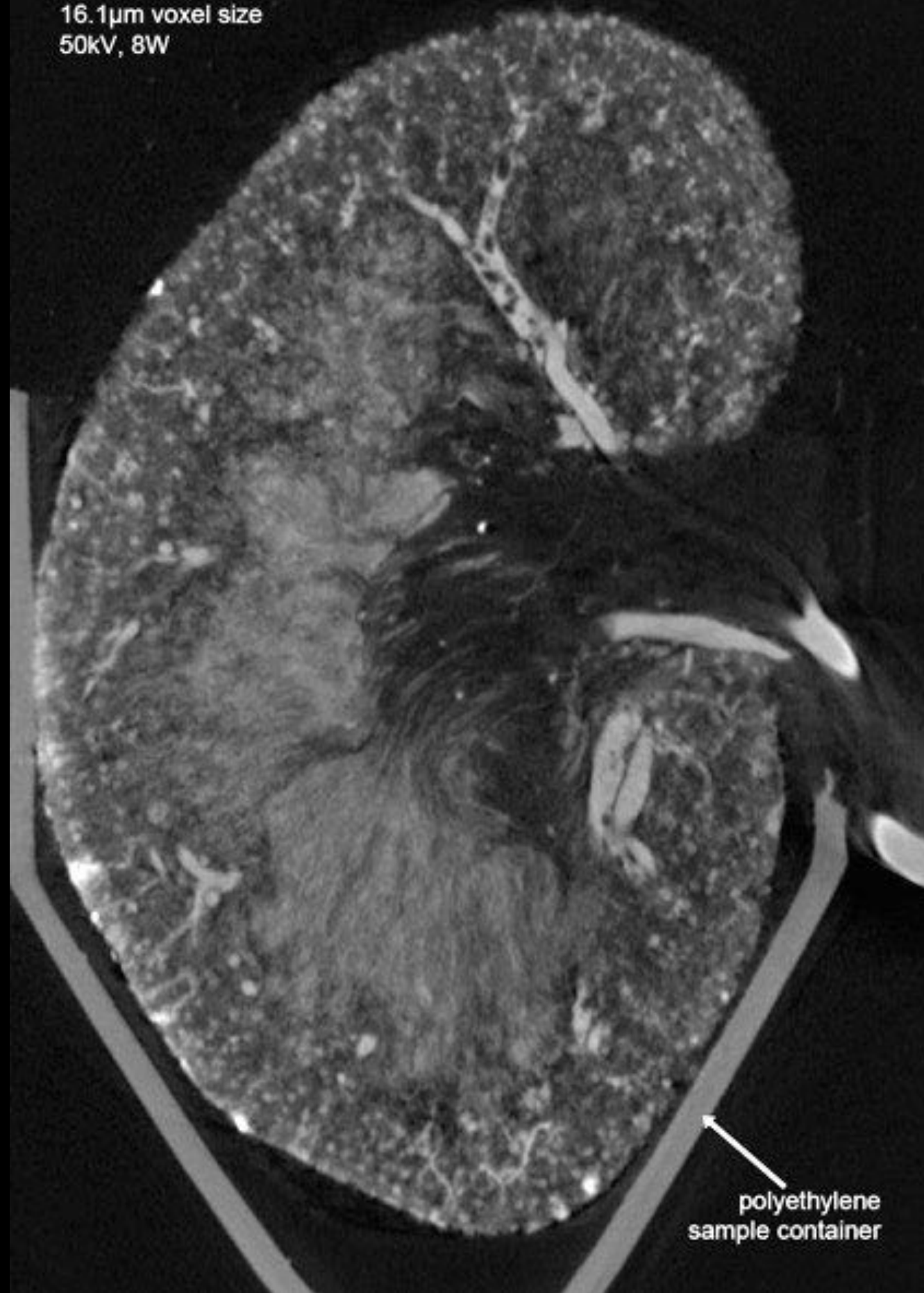


Chrysopa
(Neuroptera)
virtual sections

Worms implicated in queen's death and winglessness



Kidney1, SkyScan 1174
16.1µm voxel size
50kV, 8W



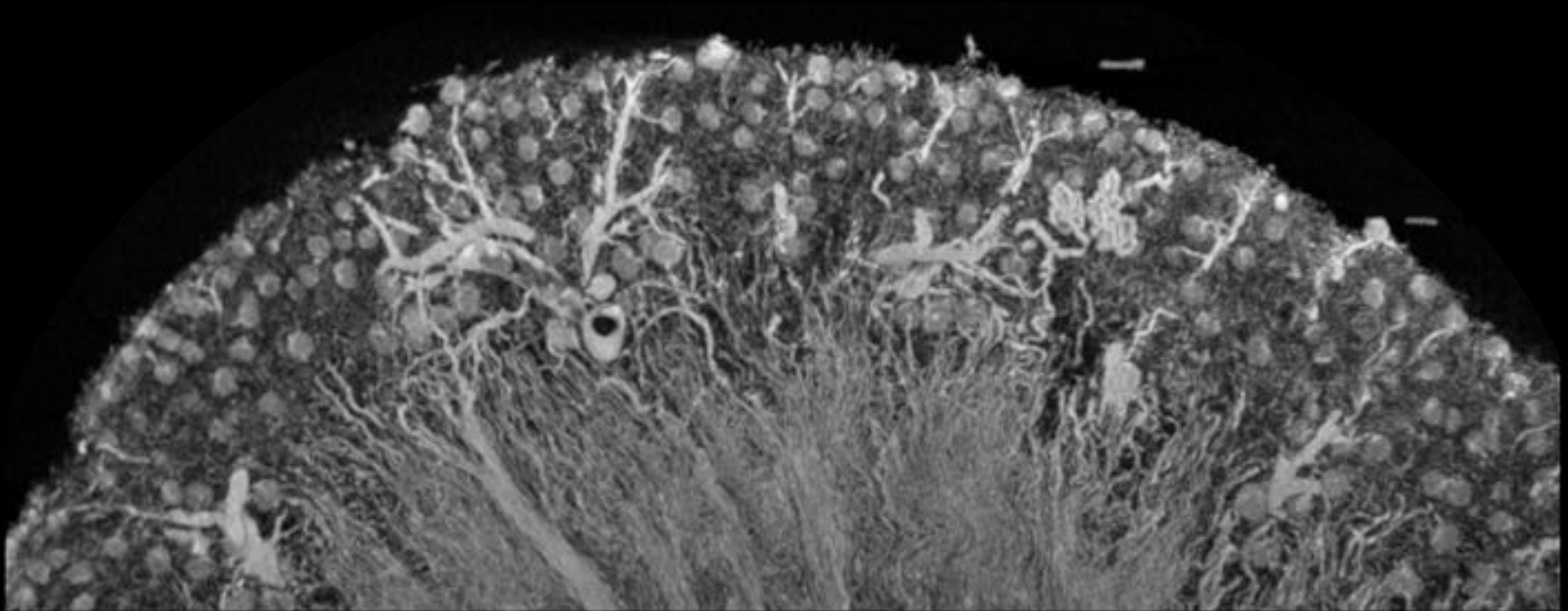
Corrosion casts of blood vessels

Rat kidney

Vascular perfusion with
Mercox polymer, tissue
removed.

Sample from Bernd Minnich,
Univ. Salzburg

Corrosion casts of blood vessels



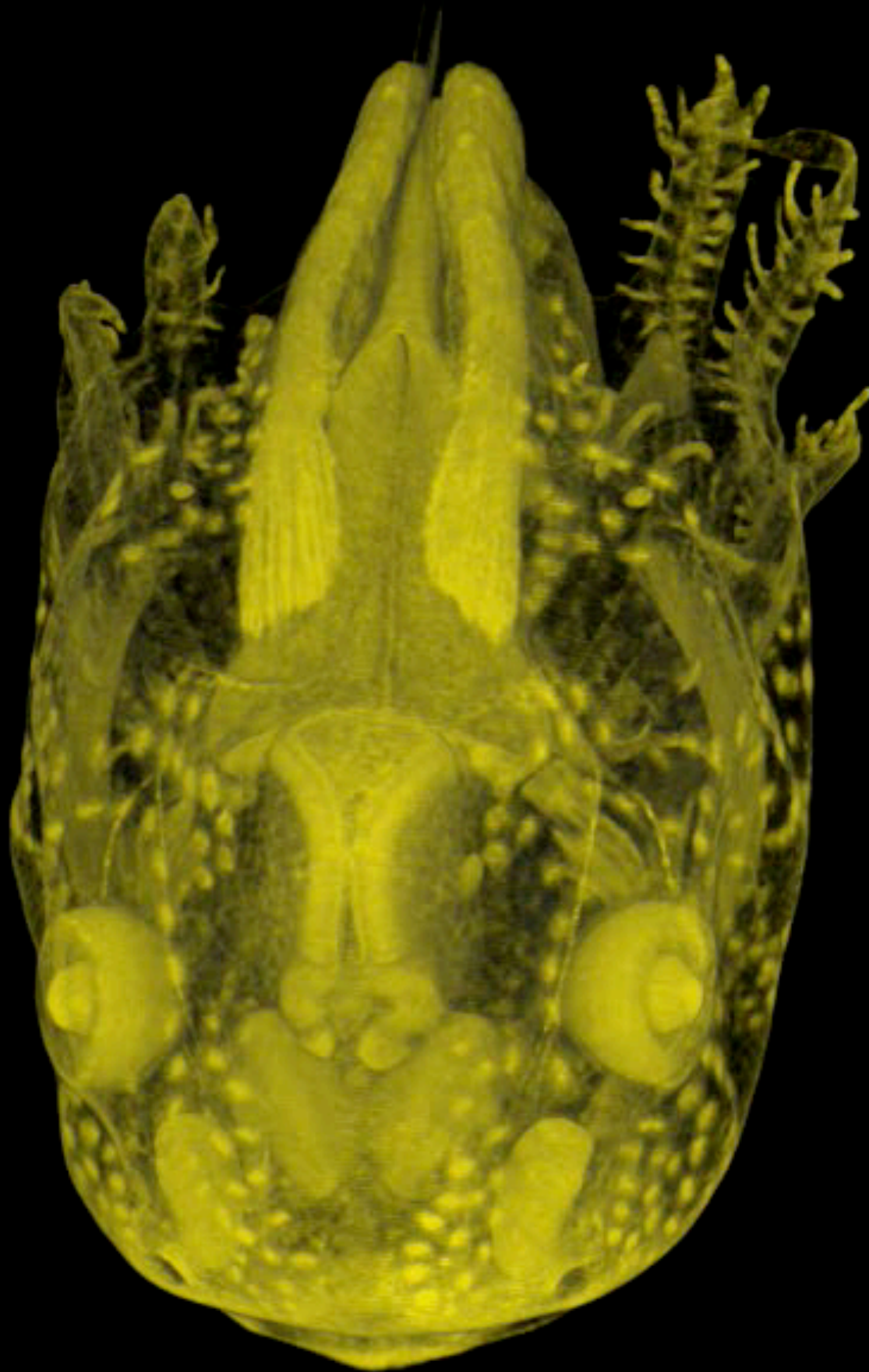
Kidney1, Xradia MicroXCT
virtual thick section
9.0 μ m voxel size

5000 μ m

Phosphotungstic acid (PTA)

0.3 - 1.0% in 70% ethanol,
or in abs. ethanol.
~1 day per mm
thickness

Axolotl feeding larva
(*Ambystoma mexicanum*)
PTA stained
9.6 μ m voxels

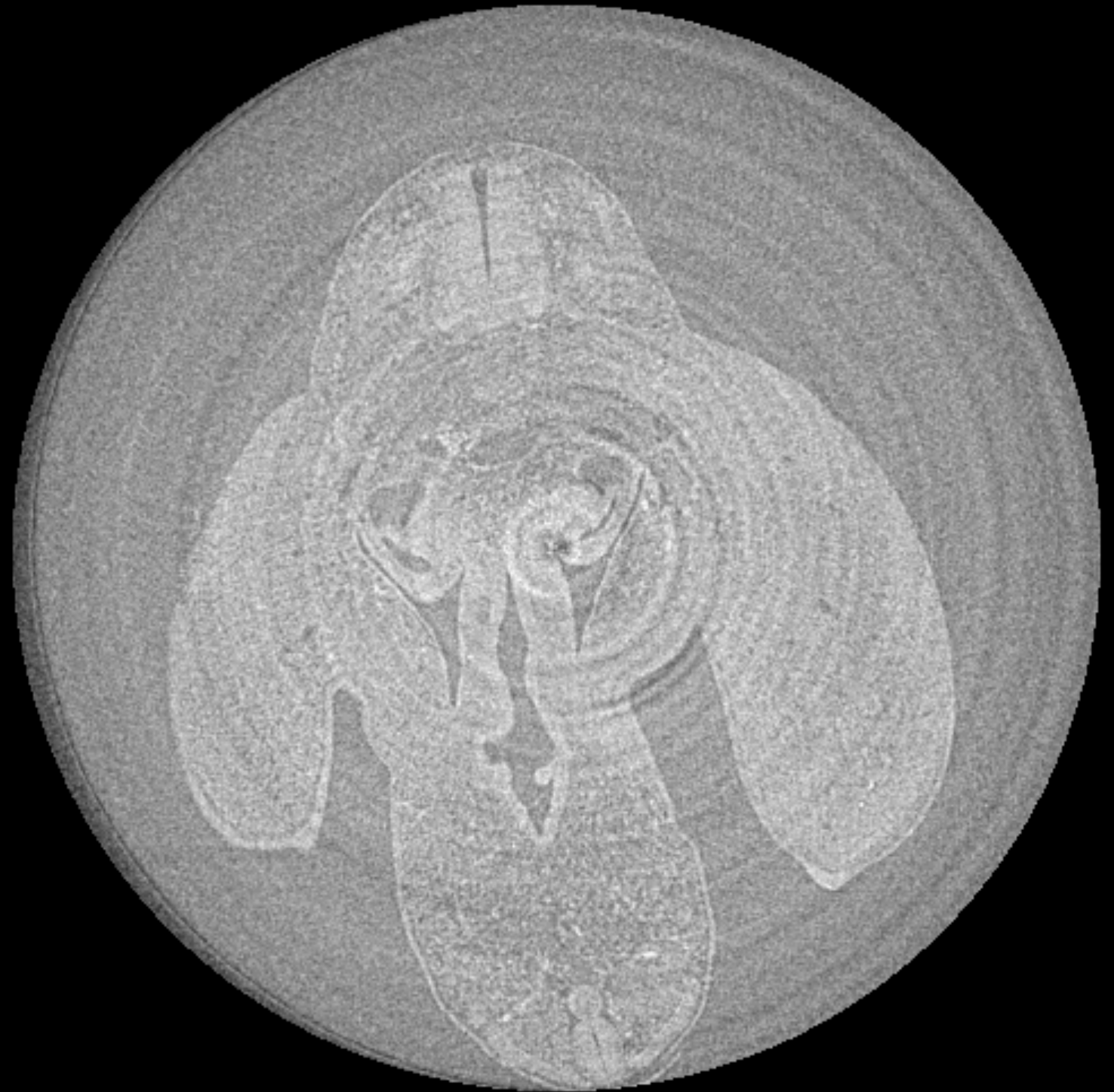


Ring artefacts

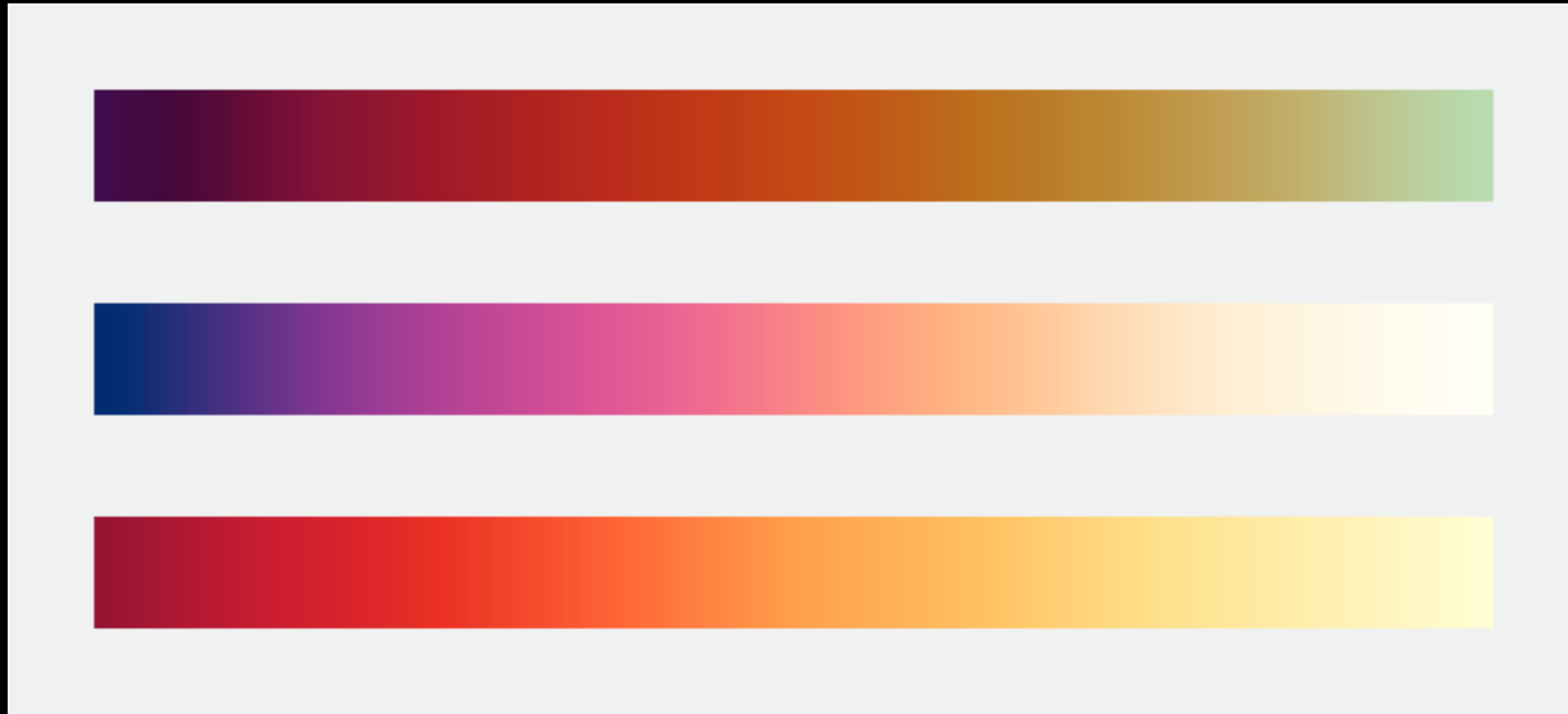
can be defeated by

- radial smoothing
- random movement

Chick embryo, unstained
early Xradia image



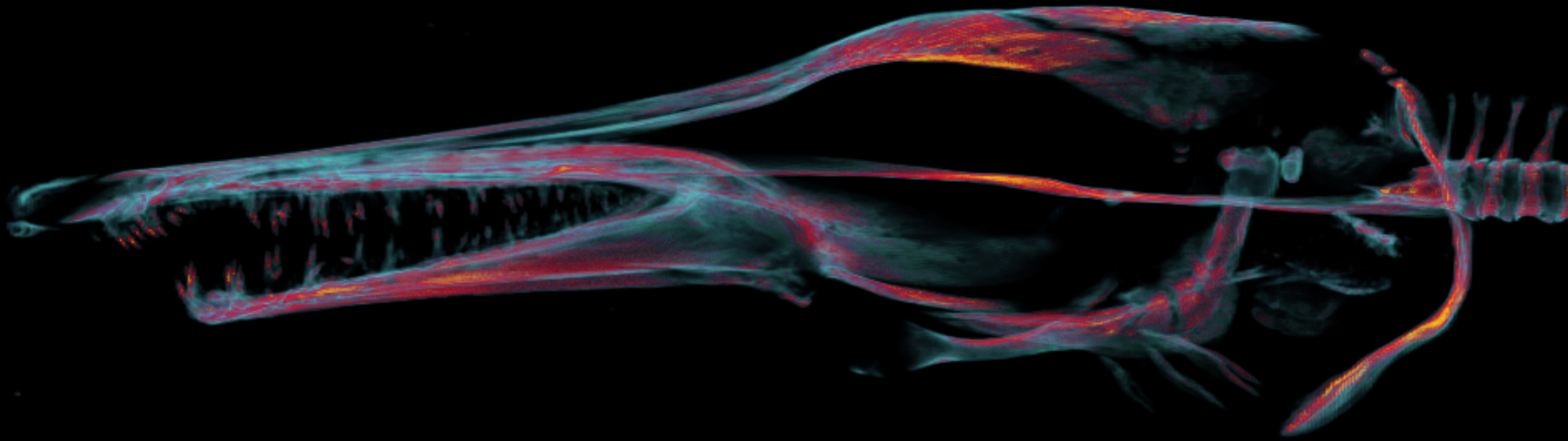
Colour palettes: eschew rainbows



<http://colorbrewer2.org>

[http://earthobservatory.nasa.gov/blogs/elegantfigures/
2013/08/06/subtleties-of-color-part-2-of-6/](http://earthobservatory.nasa.gov/blogs/elegantfigures/2013/08/06/subtleties-of-color-part-2-of-6/)

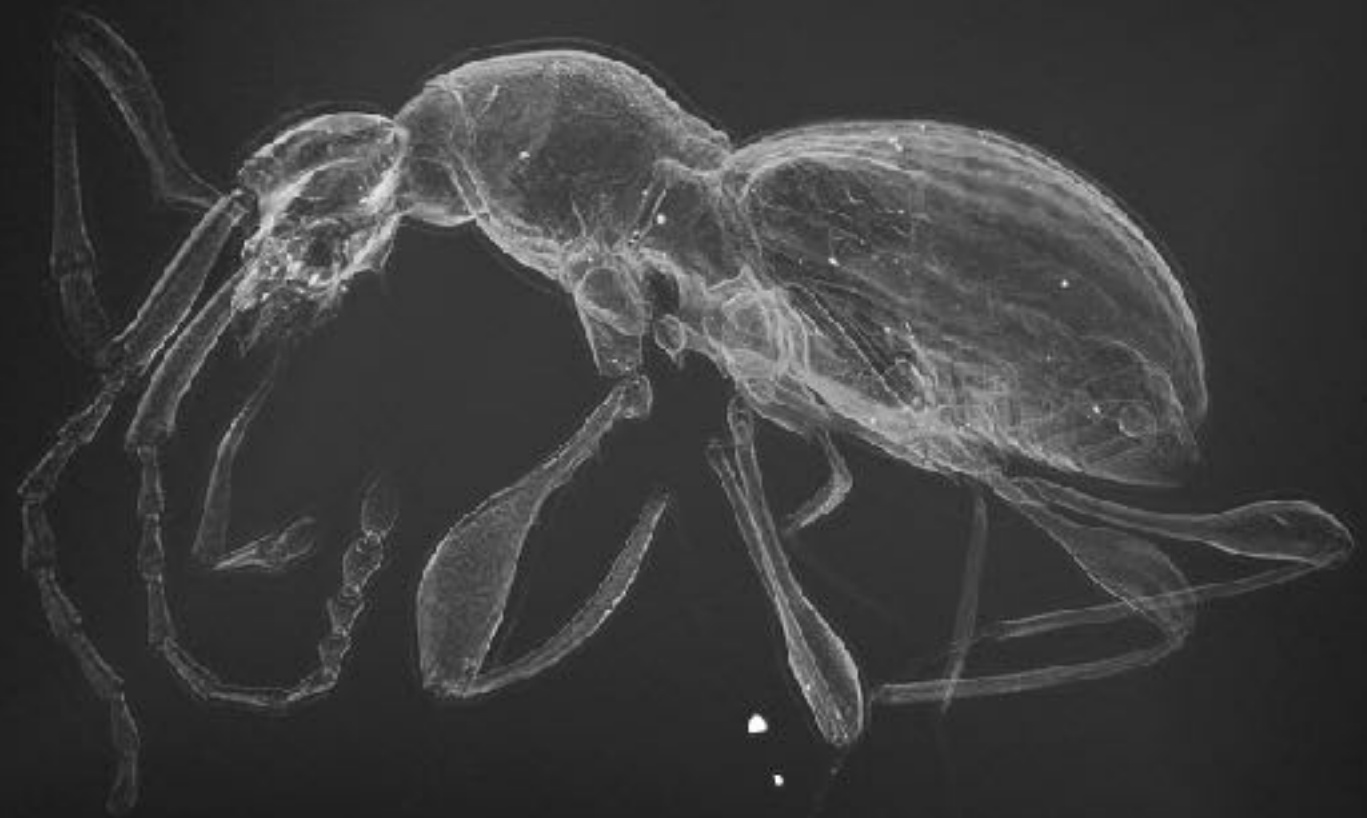
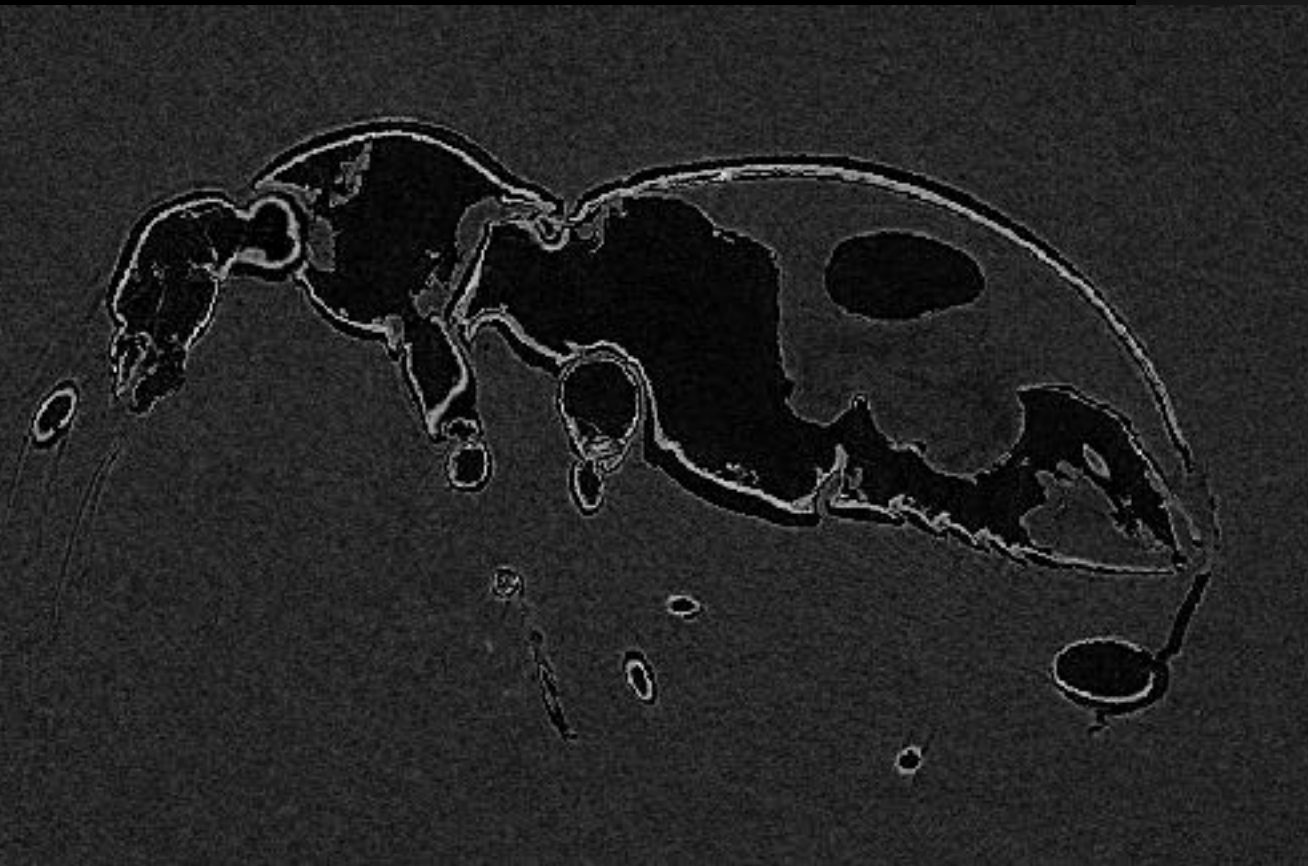
Displaying quantitative image information: bone density



Gar (*Atractosteus*)
showing relative densities
of developing bones

Fossils in amber

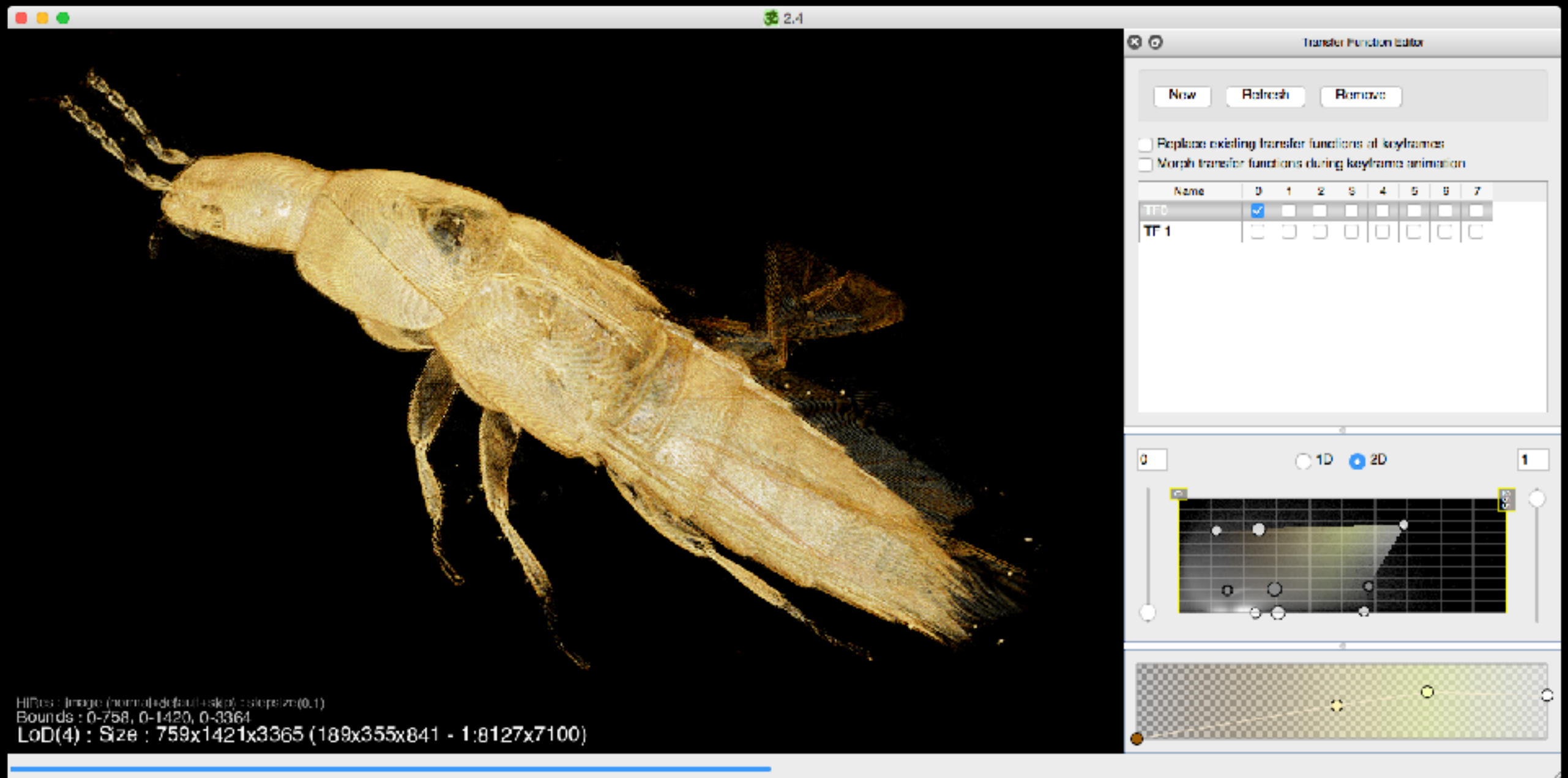
Phase helps, but much depends on preservation



Euroleptochromis.?? Work of ???

Fossils in amber

Drishti is great for visualising gradients



Staphylinid beetle. Work of Adam Brunke, NHM Vienna